PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/13, C07K 16/28, G01N 33/577, 33/68, A61K 51/10, 47/48 — A1

(11) International Publication Number: WO 98/11223

(43) International Publication Date: 19 March 1998 (19.03.98)

(21) International Application Number: PCT/EP97/04928

(22) International Filing Date: 9 September 1997 (09.09.97)

(30) Priority Data:
196 38 745.0 11 September 1996 (11.09.96) DE

(71) Applicant *(for all designated States except US)*: SCHERING AKTIENGESELLSCHAFT [DE/DE]; Patente, Müllerstrasse 178, Postfach 65 03 11, D-13342 Berlin (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MENRAD, Andreas [DE/DE]; Allerstrasse 7, D-16515 Oranienburg (DE). THIERAUCH, Karl-Heinz [DE/DE]; Hochwildpfad 45, D-14169 Berlin (DE). MARTINY-BARON, Georg [DE/DE]; Am Scheibenberg 10, D-79336 Herbolzheim-Broggingen (DE). TOTZKE, Frank [DE/DE]; Beltfoltstrasse 42, D-79089 Freiburg (DE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MONOCLONAL ANTIBODIES AGAINST THE EXTRACELLULAR DOMAIN OF HUMAN VEGF-RECEPTOR PROTEIN (KDR)

(57) Abstract

Monoclonal antibodies, secreted by hybridoma cell lines, that are directed against an epitope of the extracellular domain of human VEGF-receptor KDR, methods of determining human VEGF-receptor KDR in cell lysates or tissue analysates and the use of the antibodies in analytical assays, in diagnostics and as carrier molecules for therapeutic substances, are described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

Monoclonal antibodies against the extracellular domain of human VEGF-receptor protein (KDR)

The present invention relates to monoclonal antibodies, secreted by hybridoma cell lines, that are directed against an epitope of the extracellular domain of human VEGF-receptor KDR (kinase insert domain containing receptor), to methods of determining human VEGF-receptor KDR in cell lysates or tissue analysates and to the use of the antibodies in analytical assays, in diagnostics and as carrier molecules for therapeutic substances.

New vessels form as capillaries which sprout from existing small vessels. This process, known as angiogenesis, takes place in response to certain signals (Alberts *et al.*, Molekularbiologie der Zelle, Verlag Chemie, Weinheim).

Angiogenesis is a process that is meticulously controlled by the interaction of vascular endothelial growth factor (vascular endothelial growth factor) and its corresponding highly affine KDR-receptor on endothelial cells. KDR has been characterised as a transmembranal tyrosine kinase receptor of sub-type 5 which serves as a key regulator of vascular endothelial cell development during embryogenesis and cell regeneration (Cancer and Metastasis Reviews 15: 159-163, 1996).
Furthermore, the dysfunction of that normally meticulously regulated ligand-receptor interaction results in impairment of the angiogenesis process, which is a feature of many illnesses.
Particularly important is that the growth of tumours and their metastases has proved to be angiogenesis-dependent to a high degree.

A monoclonal antibody having a limited reaction pattern is already known. That antibody is directed against the extracellular domain of mouse KDR-homologous flk-1 and is capable of neutralising the VEGF stimulation of a chimeric flk-1/fms-receptor that is expressed in transfected 3T3 cells (Rockwell *et al.*, Molecular and Cellular Differentiation, 3 (1): 91-109 (1995)).

Monoclonal antibodies that can be used for a broad spectrum of analytical assays and methods of determining human VEGF-receptor KDR in cell lysates and tissue analysates using such antibodies have not been known hitherto.

The aim of the present invention is to provide monoclonal antibodies secreted by hybridoma cell lines. The monoclonal antibodies according to the invention can be used for a broad spectrum of analytical procedures and assays, in diagnostics and as carrier molecules for therapeutic substances.

There have now been prepared monoclonal antibodies that are directed against an epitope located within extracellular domains 1 to 7 of human VEGF-receptor KDR.

The present invention relates especially to monoclonal antibodies that are directed against an epitope located within extracellular domains 6 and 7 of human VEGF-receptor KDR.

The monoclonal antibodies AM 2-7-9, AM 2-10-1, AM 5-1-2, AM 5-10-13 and AM 2-4-1 are preferred.

The monoclonal antibodies AM 2-7-9 and AM 2-10-1 are especially preferred.

Those antibodies are directed specifically against an epitope that is located within extracellular domains 6 and 7 of human VEGF-receptor KDR.

The antibodies according to the invention exhibit a high degree of selectivity in a broad spectrum of analytical procedures and assays.

The monoclonal antibodies according to the invention have the advantage that they neither interfere with the ligand-binding domain nor activate KDR after binding.

The antibodies according to the invention are also valuable for diagnostic purposes.

For example, the monoclonal antibodies according to the invention can be used in Western blots, immunoprecipitation, ELISA, FACS analysis and in indirect immunofluoresence microscopy.

A further use of the monoclonal antibodies is in immunohistochemistry.

The monoclonal antibodies can also be used in screening for small agonistic and antagonistic molecules and in the detection of mutant receptor subtypes.

The monoclonal antibodies according to the invention can also be used in diagnostics, it being possible to couple them in combination with a suitabl contrast-enhancing substance. The antibodies react with an epitope that is not located within the ligand-binding side of the KDR and is therefore capable of reacting with all KDR-receptor populations.

A preferred contrast-enhancing substance that can be coupled to the antibodies is ^{99m} technetium.

The monoclonal antibodies according to the invention can also be used in angiogenesis-dependent phenotypes, such as tumours and metastases thereof, rheumatoid arthritis or psoriasis, or pathological symptoms resulting therefrom, when coupled with suitable toxic substances.

Suitable toxic substances are adequately known to the person skilled in the art and are described, for example, in Byers & Baldwin, Immunology (1988), 65, 329-335 and in Blakey *et al.*, Waldmann H. (ed): Monoclonal Antibody Therapy, Prog. Allergy. Basle, Karger, (1988), vol. 45, 50-90.

The invention relates also to the use of mRNA that codes for the heavy and the light chain of the antibodies according to the invention in the preparation of recombinant "single-chain antibodies".

The invention relates also to the use of the recombinant antibodies encoded by mRNA for Western blots, immunoprecipitation, ELISA.and FACS analysis, in indirect immunofluoresence microscopy and immunohistochemistry and in screening for small agonistic and antagonistic molecules and in the detection of mutant receptor subtypes.

The invention relates also to a method of determining human VEGF-receptor KDR in cell lysates or tissue analysates, characterised in that

1. the monoclonal antibodies AM 2-7-9, 2-10-1, AM 5-1-2, AM 5-10-13 or AM 2-4-1, as captor antibodies, are coupled in purified form at a concentration of 1 - 10 µg/ml in coupling buffer on ELISA plates and then excess binding sites are blocked with blocking buffer,

2. cell or tissue analysates are prepared in a suitable lysis buffer,

3. the plates are washed in washing buffer before application of the lysates,

4. the KDR-protein to be determined in the sample is quantified using a recombinant KDR-protein as standard curve,

5. the tissue and cell lysates to be analysed are then introduced into the test system,

6. incubation is carried out at room temperature for 2 hours,

7. the ELISA plates are then washed,

8. determination of the "captured" KDR is carried out by means of a polyclonal anti-KDR antiserum

and finally

9. detection by means of a chromogenic, chemiluminescent or radioactive substance is carried out.

The method can also take place in the form of a kinase test procedure that determines tyrosine-phosphorylated KDR.

In order to allow quantification of the test method, recombinant phosphorylated KDR can be used as the standard protein.

The determination of human VEGF-receptor KDR in cell lysates or tissue analysates can be carried out both qualitatively and quantitatively using the method.

The polyclonal anti-KDR antiserum used in step 8 of the method can be, for example, polyclonal anti-phosphotyrosine antiserum or a monoclonal anti-phosphotyrosine antibody.

The detection carried out in step 9 of the method can preferably be carried out with peroxidase-labelled secondary antibodies and suitable chromogenic or chemi-luminescent substrates.

The antiserum used can be, for example, from goats or rabbits or from sheep, rats or donkeys, either directly in the form of enzyme-labelled immunoglobulin or indirectly by means of enzyme-labelled anti-goat, anti-rabbit, anti-sheep, anti-rat or anti-donkey antibodies. Either alkaline phosphatase or peroxidase is used for that purpose.

The ELISA plates used in step 1 of the method can be antibody-coated and blocked.

The lysis buffer used in step 2 of the method contains 1-5 mM divalent ions and 1-15 % glycerol.

The lysis buffer preferably contains 1-2 mM divalent ions and 5-12 % glycerol.

A lysis buffer that contains 1.5 mM divalent ions and 10 % glycerol is especially preferred.

A preferred divalent ion is, for example, magnesium.

The washing and dilution buffers used in the method can be any buffers known to the person skilled in the art for that purpose.

Preferred washing buffers are those containing PBS with 0.05 % detergent and 0.1 % bovine serum albumin.

Preferred dilution buffers are those containing 1 % bovine serum albumin.

D scripti n of th Figures

Fig. 1 shows a gel with the specific reaction pattern using the example of the monoclonal antibody AM 2-10-1:

track 1: pig aorta endothelial cells, 25 µg total cell lysate
track 2: pig aorta endothelial cells, transfected with human KDR, 25 µg total cell lysate
track 3: CHO cells, transfected with human KDR, 25 µg total cell lysate
track 4: CHO cells, 25 µg total cell lysate
track 5: human umbilical cord endothelial cells, passage 6, 25 µg total cell lysate
track 6: mouse endothelial cells, 25 µg total cell lysate

Fig. 2 shows the gel for immunoprecipitation with the monoclonal antibodies AM 2-10-1 and AM 2-7-9:

track 1: 5 µg AM 2-7-9
track 2: 1 µg AM 2-7-9
track 3: 5 µg AM 2-10-1
track 4: 1 µg AM 2-10-1
track 5: isotype control, 10 µg non-specific antibody of subclass IgG1

Fig. 3 shows epitope mapping:

track 1: soluble KDR, domains 1 and 2
track 2: soluble KDR, domains 1 to 3
track 3: soluble KDR, domains 1 to 5
track 4: soluble KDR, domains 1 to 7

The following Examples describe the preparation of monoclonal antibodies according to the invention and us thereof, but the invention is not limited to these Exampl s.

1. Preparation of monoclonal antibodies

Female Balb/c mice (six weeks old) are hyper-immunised either with soluble receptor (20 µg/injection) in Freund's complete adjuvant or with bacculovirus-infected SF-9 cells (10^7 cells/injection in PBS) that express the full-length human KDR receptor protein. Specific antibody titres are determined using the extracellular KDR domain by ELISA. Three days after the last immunisation the spleen is removed. The splenocytes are fused with SP2O.Ag.14 murine myeloma cells in accordance with known methods (Köhler and Millstein, Nature 256, 495, 1975). The colonies formed are screened by means of ELISA and Western blot using the soluble extracellular KDR domain. Positive colonies are cloned three times by limited dilution, purified with protein A/Sepharose chromatography and further characterised.

2. Use in Western blots

Complete cell lysates are prepared by lysis in a suitable lysis buffer that contains the above-described concentration of divalent ions and glycerol. Equal amounts of protein are separated by means of polyacrylamide gel electrophoresis under non-reducing conditions, transferred to nitrocellulose and probed with the monoclonal antibodies AM 2-7-9 and AM 2-10-1 in accordance with procedures known *per se*. The visualisation of the immunoreactive bands is carried out either by means of chemiluminescence or enzymatically, using alkaline-phosphatase-labelled secondary antibodies and corresponding chromogenic substrates. Fig. 1 shows the gel of a Western blot.

3. Use in immunoprecipitation

Immunoprecipitations are carried out using a suitable lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% IGEPAL® CA-630, 10% (v/v) glycerol, 1.5 mM $MgCl_2$, 1 mM EGTA, 5 mM EDTA, 50 mM NaF, 2 mM sodium ortho-vanadate) and complete protease inhibitors in accordance with known methods. Fig. 2 shows the gel of the immunoprecipitation.

4. Use in immun hist chemistry

Cells are cultured on sterile cover glasses until shortly before confluence and are fixed with formaldehyde (4% v/v PBS per 4 g per litre glucose) before or after incubation with 20 µg/ml of the corresponding antibody in PBS. The immunoreaction is visualised with alkaline-phosphatase-labelled secondary antibodies and fast naphthol red as chromogenic substrate.

5. Epitope mapping

2 µg portions of recombinant soluble KDR-receptor domains that are provided with a "myc-tag" are separated by SDS-PAGE (10%) and transferred to nitrocellulose. Then two identical films are incubated with the monoclonal antibody AM 2-10-1 or with a monoclonal anti-myc antibody. Immunocomplexes are visualised by alkaline-phosphatase-labelled secondary antibodies (see Fig. 3).

Patent claims

1. Monoclonal antibodies that are directed against an epitope located within extracellular domains 1 - 7 of human VEGF-receptor KDR.

2. Monoclonal antibodies according to claim 1, characterised in that the anti bodies are directed against an epitope located within extracellular domains 6 and 7 of human VEGF-receptor KDR.

3. Monoclonal antibodies according to claim 2, characterised in that they are the antibodies AM 2-7-9, AM 2-10-1, AM 5-1-2, AM 5-10-13 and AM 2-4-1.

4. Monoclonal antibodies according to claims 2 and 3, characterised in that they are the antibodies AM 2-7-9 and AM 2-10-1.

5. Use of the monoclonal antibodies according to claims 1 to 4 in Western blots, immunoprecipitation, ELISA, FACS analysis and in indirect immunofluorescence microscopy.

6. Use of the monoclonal antibodies according to claims 1 to 4 in immuno histochemistry.

7. Use of the monoclonal antibodies according to claims 1 to 4 in screening for small agonistic and antagonistic molecules and in the detection of mutant receptor subtypes.

8. Use of the monoclonal antibodies according to claims 1 to 4 in diagnostics.

9. Use according to claim 8, characterised in that the monoclonal antibodies are coupled in combination with a suitable contrast-enhancing substance and are used in that form.

10. Use according to claims 8 and 9, characterised in that the contrast-enhancing substance coupled to the antibody is ^{99m} technetium.

11. Use of the monoclonal antibodies according to any one of claims 1 to 4 coupled to suitable toxic substances.

12. Use according to claim 11 in angiogenesis-dependent phenotypes, such as tumours, rheumatoid arthritis or psoriasis.

13. Use according to claims 11 and 12 in the case of metastases.

14. Use of mRNA that codes for the heavy and the light chain of the antibodies according to the invention in the preparation of recombinant "single-chain antibodies".

15. Use of the recombinant antibodies encoded by mRNA according to claim 14 in Western blots, immunoprecipitation, ELISA, FACS analysis, in indirect immunofluorescence microscopy and in immunohistochemistry.

16. A method of determining human VEGF-receptor KDR in cell lysates or tissue analysates, characterised in that

1. the monoclonal antibodies AM 2-7-9, 2-10-1, AM 5-1-2, AM 5-10-13 or AM 2-4-1, as captor antibodies, are coupled in purified form at a concentration of 1 - 10 µg/ml in coupling buffer on ELISA plates and then excess binding sites are blocked with blocking buffer,

2. cell or tissue analysates are prepared in a suitable lysis buffer,

3. the plates are washed in washing buffer before application of the lysates,

4. the KDR-protein to be determined in the sample is quantified using a recombinant KDR-protein as standard curve,

5. the tissue and cell lysates to be analysed are then introduced into the test system,

6. incubation is carried out at room temperature for 2 hours,

7. the ELISA plates are then washed,

8. determination of the "captured" KDR is carried out by means of a polyclonal anti-KDR antiserum

and finally

9. detection by means of a chromogenic, chemiluminescent or radioactive substance is carried out.

17. Method according to claim 16, characterised in that the method is carried out in the form of a kinase test procedure that determines tyrosine-phosphorylated KDR.

18. Method according to claims 16 and 17, characterised in that recombinant phosphorylated KDR is used as the standard protein.

19. Method according to claims 16 to 18, characterised in that the polyclonal anti-KDR antiserum used in step 8 of the method is polyclonal anti-phosphotyrosine antiserum or a monoclonal anti-phosphotyrosine antibody.

20. Method according to claims 16 to 19, characterised in that the substance used for detection in step 9 of the method is a peroxidase-labelled secondary antibody with suitable chromogenic or chemiluminescent substrates.

21. Method according to claims 16 to 20, characterised in that the lysis buffer used in step 2 of the method contains from 1 to 5 mM divalent ions and from 1 to 15 % glycerol.

22. Method according to claims 16 to 20, characterised in that the lysis buffer used in step 2 of the method contains from 1 to 2 mM divalent ions and from 5 to 12 % glycerol.

23. Method according to claims 16 to 20, characterised in that the lysis buffer used in step 2 of the method contains 1.5 mM divalent ions and 10 % glycerol.

24. Method according to claims 21 to 23, characterised in that the divalent ion is magnesium.

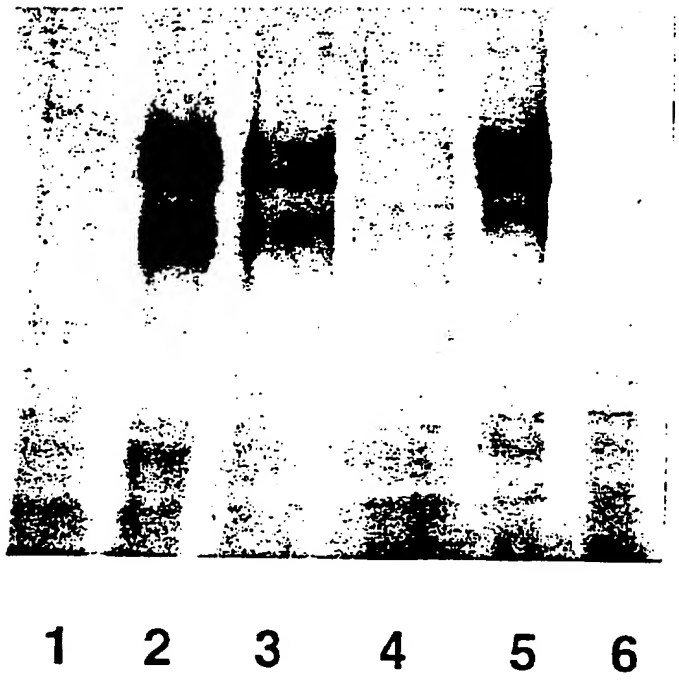
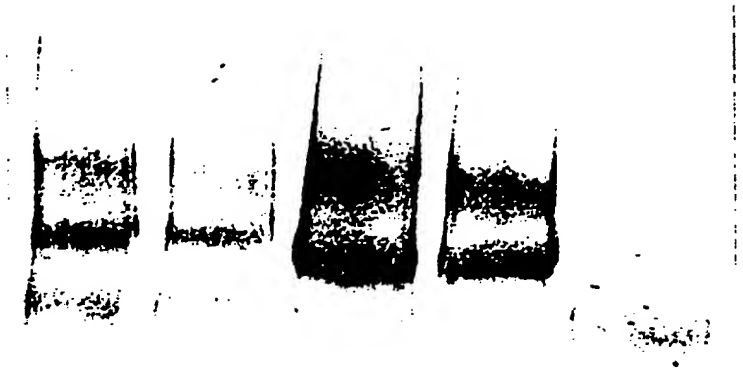


Fig. 1



1 2 3 4 5

Fig. 2

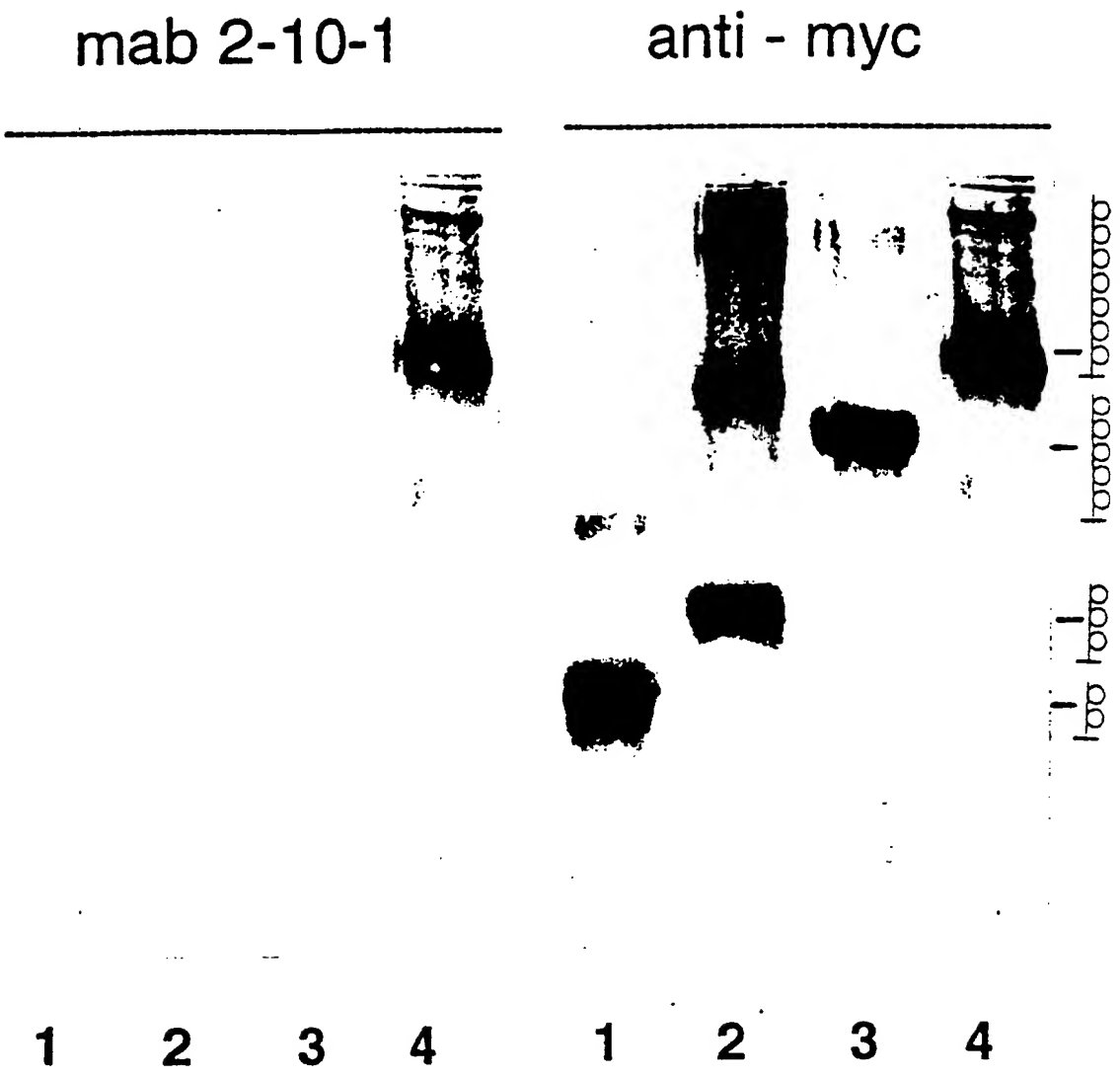


Fig. 3

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 97/04928

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/13 C07K16/28 G01N33/577 G01N33/68 A61K51/10
A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 21868 A (IMCLONE SYSTEMS INC.) 17 August 1995 see page 37, line 15 - page 38, line 15 see claims	1,5-15
X	P. ROCKWELL ET AL.: "In vitro neutralization of vascular endothelial growth factor activation of flk-1 by a monoclonal antibody." MOLECULAR AND CELLULAR DIFFERENTIATION, vol. 3, no. 1, 1995, pages 91-109, XP002052455 cited in the application see the whole document	1,5-15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 1998	17.02.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern. ial Application No
PCT/EP 97/04928

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. ROCKWELL ET AL.: "Anti-tumor effects of a neutralizing monoclonal antibody to the tyrosine kinase receptor flk-1." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, vol. 36, March 1995, USA, page 425 XP002052456 see abstract 2534 ---	1,5-15
A	B. TERMAN ET AL.: "Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor." BI CHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 187, no. 3, 30 September 1992, DULUTH, MN, USA, pages 1579-1586, XP002052457 see abstract see figure 1 see page 1582, line 25 - line 37 ---	1-24
A	J. WALTENBERGER ET AL.: "Different signal transduction properties of KDR and flt1, two receptors for vascular endothelial growth factor." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 43, 28 October 1994, BALTIMORE, MD, USA, pages 26988-26995, XP002052458 see page 26989, left-hand column, line 17 - line 19 ---	1-24
A	B. MILLAUER ET AL.: "High affinity VEGF binding and developmental expression suggest flk-1 as a major regulator of vasculogenesis and angiogenesis." CELL, vol. 72, 26 March 1993, CAMBRIDGE, MA, USA, pages 835-846, XP002052459 see page 842, left-hand column, line 1 - line 5 see figure 1 ---	1-24
A	WO 94 11499 A (MAX PLANCK GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.) 26 May 1994 see page 38, line 28 - page 39, line 2 see claims --- -/--	1-24

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 97/04928

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	A. MENRAD ET AL.: "Novel antibodies directed against the extracellular domain of the human VEGF-receptor type II." HYBRIDOMA, vol. 16, no. 5, 1997, NEW YORK, NY, USA, pages 465-471, XP002052460 see the whole document -----	1-24

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/04928

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3 [ ] Claims Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INF RMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 11-13 (partially, as far as an in vivo method is concerned) are directed to a method of treatment of the human/animal body , and although claims 8-10 (partially, as far as an in vivo method is concerned) are directed to a diagnostic method practised on the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interm: .al Application No
PCT/EP 97/04928

Patent document cited in search report	Publication date	Patent family member(s)	Publication . date
WO 9521868 A	17-08-95	AU 1914795 A	29-08-95
		EP 0741748 A	13-11-96
WO 9411499 A	26-05-94	AU 5562794 A	08-06-94
		CA 2149298 A	26-05-94
		CN 1094445 A	02-11-94
		EP 0669978 A	06-09-95
		JP 8505763 T	25-06-96